A Note on Drosophila as a Mutagenicity Test System

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By virtue of its considerable genetic versatility, *Drosophila melanogaster* may be used to detect, in the entire array of germ cell stages, a wide spectrum of chemically induced genetic alterations, including those with severe or obvious effects, such as dominant lethals, chromosome loss, complete and mosaic translocations, recessive lethals, and "visibles", and nondisjunction, as well as those causing only mildly detrimental effects but which in aggregate may be as harmful as mutations with severe effects (*1–3*). Since the former are far more readily detected than the latter, and include the major types of genetic damage responsible for abortions, diseases, abnormalities, and malfunctionings in humans, they are as a group, of obvious primary concern in any mutagenicity testing program.

The two most widely used procedures used in *Drosophila* screening for genetic alterations in postmeiotic, meiotic, and premeiotic cells (and in existence now for more than four decades) are the sex-linked recessive lethal test and the translocation test, often combined, in practice, in the same experiment [see Abrahamson and Lewis (*4*) for details of procedure]. The sex-linked recessive lethal test detecting recessive mutations (associated or not with chromosome aberrations) arising in the X chromosome of male germ line cells, is probably the most utilitarian in the battery of procedures available in *Drosophila* for mutagenicity testing. Reasons are (a) scoring for lethals can be made with relative ease and considerable precision, the precision increasing when putative lethals undergo retesting, the latter itself a relatively simple procedure; (b) whereas all genes in the X chromosome may not give rise to a lethal condition when hemizygous for a "null" allele, by reducing this number to as low as 20% of the estimated 1000 genes in the X chromosome leaves some 200 to be tested, in the same experiment, for recessive lethals [extrapolation to the figure of 1000 genes derives most recently from the work of Judd et al. (*5*); (c) the spontaneous rate of recessive lethals in the X chromosome is relatively low (about 0.1–0.2%); and (d) testing the X chromosome in the male obviates the problem of pre-existing lethals, since the male must be free of X chromosome lethals to serve in the P1 generation. The translocation test, monitoring the occurrence of interchanges between nonhomologous chromosomes in the male germ line, is used as a means of determining the potential of a chemical to induce chromosome breaks capable of rejoining and of being transmitted to the next generation as balanced chromosome aberrations. The rationale for the choice of this test is based on the following considerations. (a) Relatively straightforward genetic procedures permit detection of translocations between chromosomes 2 and 3, between the Y chromosome and chromosome 2, the Y

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chromosome and chromosome 3, and translocations involving all three chromosomes simultaneously; thus a major portion of the genome may be assayed for translocations in the same experiment (only the X chromosome and the small fourth chromosome are not included in the testing). (b) The spontaneous rate of translocations in *Drosophila* is very low, probably less than 10^{-4}. (c) Translocations arise only following chromosome breakage, so that their recovery provides unequivocal evidence of the break-inducing ability of the chemical. Employment of a third procedure, one testing for nondisjunction of chromosomes, a genetic event of considerable importance in humans, which may be caused by nongenetic factors (e.g. spindle failures, chromosome stickiness) becomes particularly desirable where the chemical under investigation fails to show evidence of mutagenic activity as judged by the results of the recessive lethal and translocation tests. Whereas in the past only nondisjunction of the X chromosomes in the female or the X and Y chromosomes in the male could be scored with ease and precision, it is now relatively simple to follow, by genetic means, nondisjunction of the small fourth chromosome in conjunction with nondisjunction of the sex chromosomes (the former making use of the special compound fourth chromosome). In this way, events paralleling in principle those leading to Turner's (XO) or Klinefelter's (XXY) syndrome, and to group G-21 trisomy (Down's syndrome) in humans may be monitored in the same experiment.

Briefly, then, these procedures may be used to detect three important classes of genetic alterations: recessive lethals and translocations representing transmissible alterations associated with both short-term and long-term deleterious effects, and nondisjunction leading to lethality of monosomic and trisomic offspring or perhaps, of greater significance to the health and economy of the population, to impairments in surviving offspring. It should be noted, further, that these type of genetic alterations are either not testable or testable only with considerable difficulty in germ cells of mammalian systems.

The question arises as to how these procedures can most usefully be exploited in evaluating the potential mutagenic hazard of a compound. Three ways may be described. The simplest, although not always the most informative, is to administer the compound directly "off the shelf", by feeding, microinjection or by vacuum injection (*6*). However, under these circumstances, whereas a positive response to the compound raises the possibility that the compound may be mutagenic in humans, a negative response could be due to the absence in *Drosophila* of the appropriate metabolic mechanism required to change the compound from a nonmutagenic to a mutagenically active form. To deal with this possible shortcoming, it would be desirable to use *Drosophila* in conjunction with experiments employing the conventional mammalian host mediated assay or microsomal activating systems (*7*). Thus, for each compound investigated, results from the *Drosophila* tests and those from the rodents would be evaluated, with special attention given to a comparison of the response of *Drosophila* to the compound, and the *in vitro* and *in vivo* responses of the indicator bacteria in the host mediated assay. The desirability of subsequently using *Drosophila* as an indicator organism in the host mediated assay to complement the bacterial system woild be suggested if the compound produces (a) no mutagenic effect in *Drosophila* or in bacteria *in vitro* but a positive effect in bacteria *in vivo*, or (b) a mutagenic effect in *Drosophila* and in bacteria *in vitro* but not in bacteria *in vivo*. The former suggests that the mammalian host system causes an enhancing effect, the latter suggests a detoxifying effect on the mutagenic activity of the compound (*8*). It should be noted that Lee et al. (*9*) and Gee et al. (*10*), using blood plasma or other tissues from mutagen-treated hamsters, and Browning (*11*), using intraperitoneal fluid from mice, have demonstrated the utilizability of *Drosophila* as an indicator organ-

ism in mammalian host-mediated assay systems.

Finally, in view of the findings just noted, it should not be long before *Drosophila* (preferably in conjunction with the conventional mouse host mediated assay or microsomal activating systems) is used to test for the possible mutagenic action of compounds found in humans, for example, therapeutic drugs, by assessing the mutation-inducing ability of drug-containing body fluids taken directly from the individuals receiving these drugs.

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